

Disposable parallel poly(dimethylsiloxane) microbioreactor with integrated readout grid for germination screening of *Aspergillus ochraceus*

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In this work a disposable, parallel microbioreactor (MBR) suitable for screening in batch or continuous mode is presented. The reactor consists of five parallel microchambers made of poly(dimethylsiloxane) bonded to a glass substrate. A grid structure is engraved on each chamber, allowing subsequent morphology imaging. Measurements are recorded over the entire cultivation period with constant parameters, namely, position and focus in the z-axis. The microdevice may be used for either parallel, uni- or multiparametric screening, and overcomes the drawback of gridless microwell plates which require expensive equipment such as an inverted microscope with an automatic stage. To validate the scalability from laboratory scale to microscale, and thus the cultivation protocol in the MBR, the germination of fungal spores (*A. ochraceus*) is evaluated for two different key magnitudes (*pH* and temperature) and compared to the results obtained from conventional laboratory scale systems (flasks and agar plates). Information on germination capacity with regard to interspecies' variability allows for optimization of industrial processes as optimal *pH* and temperature matched to the mesoscopic cultivation systems. The germination conditions therefore remain unaffected inside the MBR, while providing the following advantages: (i) dramatic reduction of medium consumption, (ii) submerged cultivation with constant oxygen supply, (iii) assured low cost and disposability, and (iv) possibility of a continuous cultivation mode. © 2011 American Institute of Physics. [doi:10.1063/1.3553004]

I. INTRODUCTION

Filamentous fungi of the *Aspergillus* species present one of the most important host organisms for the production of various organic acids, enzymes, and antibiotics. Furthermore, they are known for their capability of biotransformation when being used as catalysts for the production of active pharmaceutical ingredients.^{1–4} In this way, spores are applied as starting cultures (inocula) in submerged cultivation processes. In all applications in the environmental, food, or pharmaceutical industry, reliable and reproducible inoculation conditions and quality of fungi spores in the inocula, which primarily grow to active biomass in the course of germination, are of high importance. Germination capacity and hyphal growth represent reliable quality indicators of interspecies' variability and may therefore be used for optimization of industrial processes. Given its importance as a developmental stage during the life cycle of fungi, spore germination has received extensive interest not only for physiologists and seed technologists, but also for ecologists.⁵

As reported by Griffin,⁶ the germination behavior comprises three subsequent steps. (1) For the “activation” of the resting spore, appropriate environmental conditions are required, such as oxygen, water, and carbon dioxide. (2) During “isotropic growth,” spores swell due to water

uptake, wall growth, and the resumption of numerous metabolic activities.^{6,7} (3) “Polarized growth” results from wall deposition forming a germ tube, which converts into an elongating and branching mycelium.

Although germination characteristics are generally known, it is still a challenge to optimize the germination conditions for submerged cultures that guarantee optimal posterior cultivation results, since they are highly species dependent. Parameters such as initial spore concentration, pH, medium composition, and temperature influence the germination performance and have to be carefully screened. Ranal *et al.*⁵ reviewed the different measurement parameters for evaluation of the germination process that had already been presented in literature. One qualitative parameter is the germination capacity, which is based on a binary answer (germinated/nongerminated), and commonly converted in a quantitative attribute (with percentage as units).

Over the past decades, a lot of research has been conducted to better understand the influence of process conditions during cultivation of filamentous fungi. However, the influence of spore quality in seeding cultures has not been investigated in depth. This is partially due to the lack of applicative methods to validate spore posterior performance during submerged cultivation processes before their cultivation by noninvasive characterization. The challenge of using down-scaled instruments to predict germination behavior in scaled up reactors is to assure that process conditions are as similar as possible. Some of the requirements for germination screening include the following: (i) cultivation under submerged conditions, (ii) sufficient oxygen supply during germination process, (iii) noninvasive imaging of spore germination (even demanding adhesion of the spores to a substrate), (iv) low cost (being utilized as disposables and no need for bulky and expensive equipment such as an inverted microscope with automatic stages), and (v) easy and rapid handling. So far, evaluation of germination has been carried out in moistened dishes, flasks, in microwell plates, as well as onto agar plates. These tools have been successfully proven; however, an optimization of these screening instruments can still be improved to fulfill all mentioned requirements.

Abdel-Rahim *et al.*⁸ characterized the spores of *Aspergillus niger* onto clean sterile glass slides placed in moist paper-lined dishes. The germination process was suppressed in 3 h intervals by addition of lactophenol cotton blue stain and observed microscopically. Paul *et al.*⁹ cultivated the spores of *Penicillium chrysogenum* under submerged conditions in flasks. Germination was stopped by use of cotton blue in lactophenol after 4 h. In comparison to Abdel-Rahim’s work, Paul *et al.* developed an automated imaging analysis method to evaluate the germination behavior. Imaging was carried out after filling the diluted spore sample into Helber counting chambers. These chambers were purchased explicitly without grid lines to ease the image processing. Solid agar plates are another possibility to assess the germination characteristics and the viability of spores as reported by Araujo and Rodrigues,¹⁰ Rodríguez Porcel *et al.*,¹¹ and Pardo *et al.*¹² One drawback of applying solid agar, however, is that germination does not take place under submerged conditions resulting, e.g., in varying nutrient supply. Thus, this method does not reveal the same conditions that exist during submerged cultivation processes.

Oh *et al.*¹³ reported on a morphological recognition of fungal spore germination by a computer-aided image analysis within a multiwell culture chamber. Before inoculation, the glass surface was coated with poly-L-lysine in order to obtain an adhesion of the spores to the bottom of the wells. Although this assures continuous observation of germinating spores under submerged conditions, it leads to a missing oxygen supply during germination, since the screening system is made of glass.

A step toward integration has already been completed whereby an ultrawide field cell monitoring array platform based on shadow imaging^{14,15} has been added. This configuration has proved able to make phenotype characterization of both cells and particles, but there has still been no results concerning the viability of such approaches for germination screening.

To summarize, the supply of noninvasive screening tools that ensure cultivation conditions as similar as possible to up-scale processes is challenging. However, the recent attempts to merge microfluidic technologies with biological and biotechnological analysis have recently evolved into a promising research field due to its numerous advantages.¹⁶ A myriad of different microsystems

ranging from drop generators to single-cell cultivation systems have been developed.^{17–19} The great potential lies in the ease of fabrication of microfluidic chambers with integrated online analytics mostly based on the inexpensive material poly(dimethylsiloxane) (PDMS). PDMS is a commercially available, two component polymer with silicone-like consistency. It is a flexible polymer with interesting properties, including its complete biocompatibility and nontoxicity, its high transmittance from the visible to the near infrared, and its low cost and technological simplicity.²⁰ Transparency is inalienable for any microscopic observation. Moreover, PDMS shows permeable characteristics to gases and thus allows continuous oxygen penetration through the PDMS reactor membrane.²¹ Due to the various advantages of PDMS-based microdevices for the cultivation of cells, the polymeric material is very convenient and widely used.^{22–24}

We present the design and fabrication procedure of a parallel microbioreactor device containing five equal reaction chambers. The peculiarity of each chamber is its soft lithographic engraved grid structure that is realized by a two-lithographic step. It allows uni- or multiparametric morphology screening (via microscopy). Uniparametric measurement is related to quintuple measurements under similar conditions, whereas multiparametric screening refers to cultivations with the same inoculum but five different cultivation parameters used simultaneously. Due to the implemented grid structure, the same locations and thus the same spores with the same focus in z-axis can be subsequently recorded in a defined interval over the entire cultivation time. The completely transparent microbioreactor chip can be used in order to examine the isotropic growth (swelling) and the polarized growth in microliter scale under submerged cultivation conditions. Identical germination conditions (e.g., same inoculum) are assured within the five parallel microchambers heading toward only one altered screening parameter.

Compared to microwell plates, however, an inverted microscope is not needed for a zoom factor of 20 as the total thickness of the chip is less than 2 mm, featuring a height of 230 μm and thus allowing focus adjustment in the z-axis with a normal microscope. In addition, germination screening in well plates is generally only performed when an inverted microscope with an automatic stage system is available. This configuration hampers the low cost goal, which limits its applicability to research centers, without high impact in on-site applications (as could be point of care analysis). Due to their inexpensive materials and fabrication technology, the produced microdevices can be applied as disposable tools. To validate the down-scalability from laboratory scale to microscale, and thus the cultivation protocol in the microdevice, the germination of fungal spores (*A. ochraceus* as model organism) is evaluated for two different process parameters (pH and temperature) and compared to the results obtained from conventional laboratory scale system (flasks and agar plates).

II. DESIGN OF THE ENGRAVED PARALLEL MICROBIOREACTOR DEVICE

The developed microbioreactor device ($21 \times 39 \text{ mm}^2$) consists of five identical, parallel reactor chambers (total filling volume of 9 μL each) with fluidic inlet and outlet channels (Fig. 1, left). Each of the five reactor chambers features five grid arrays uniformly distributed over the reactor chamber and labeled consecutively in Roman letter numbers I–V (Fig. 1, top right). The grid matrix consists of grid rows and grid columns, labeled from A to I and from 1 to 9, respectively. Each square features a side length of 100 μm . This allows for easy focusing and imaging for spore counting or morphology studies at different locations over defined time intervals.

III. MATERIALS AND METHODS

A. Fabrication of the microbioreactor device

The fabrication of the microfluidic biodevice is based on UV-depth lithography using a negative photoresist (SU-8) and soft lithography applying PDMS for molding. The negative master structure is processed via a double photolithographic process. A chromium-gold layer is sputtered on a soda-lime glass substrate (Borofloat®33, Berlin, Germany) and structured photolithographically by means of ma-P 1215 (micro resist technology GmbH, Berlin, Germany) with alignment structures [Fig. 2(a)/Fig. 2(b)]. Then, the top side of the substrate is spun-coated with SU-8 5

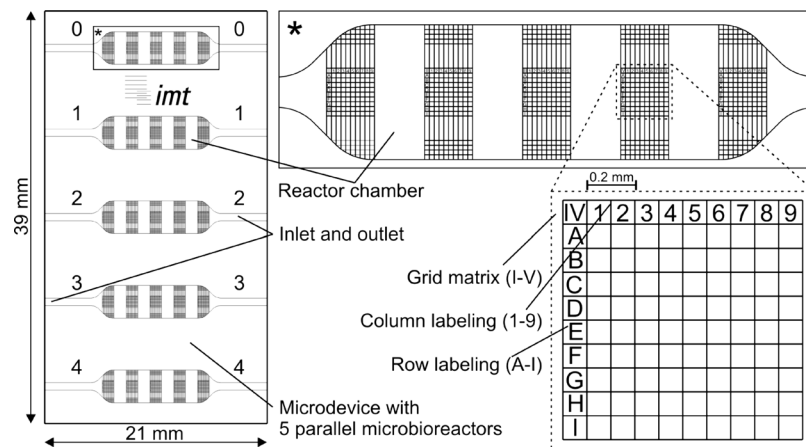


FIG. 1. Schematic of the microfluidic device design consisting of five parallel reactor chambers (left), one single reactor chamber with the five implemented grid arrays (top right), and one grid array with equally distributed grid columns and grid rows (bottom right).

(MicroChem Corp., Newton, MA, USA) at 2500 min^{-1} for 30 s and dried for 10 min at 95°C . This layer, acting both as an adhesion promoter and seed layer for the following negative structure SU-8 layer, is flood exposed to UV-light and baked at 95°C for 10 min [Fig. 2(c)]. Afterward, SU-8 50 is spun at 1200 min^{-1} , leveled and dried at 95°C for 2 h. The same process step is repeated, including additionally 1.5 h of drying, in order to attain a total layer thickness of $230 \mu\text{m}$. The first structure layer is exposed to UV-light for 100 s by aligning the first chromium reactor mask [Fig. 2(d)]. After the postexposure bake for 45 min at 95°C , a final layer of SU-8 5 is spun-on at a rotational speed of 3000 min^{-1} (for the grid mask), baked for 20 min at 95°C , and exposed to UV-light for 10 s [Fig. 2(e)]. In doing so, grid heights between 5 and $10 \mu\text{m}$ can be

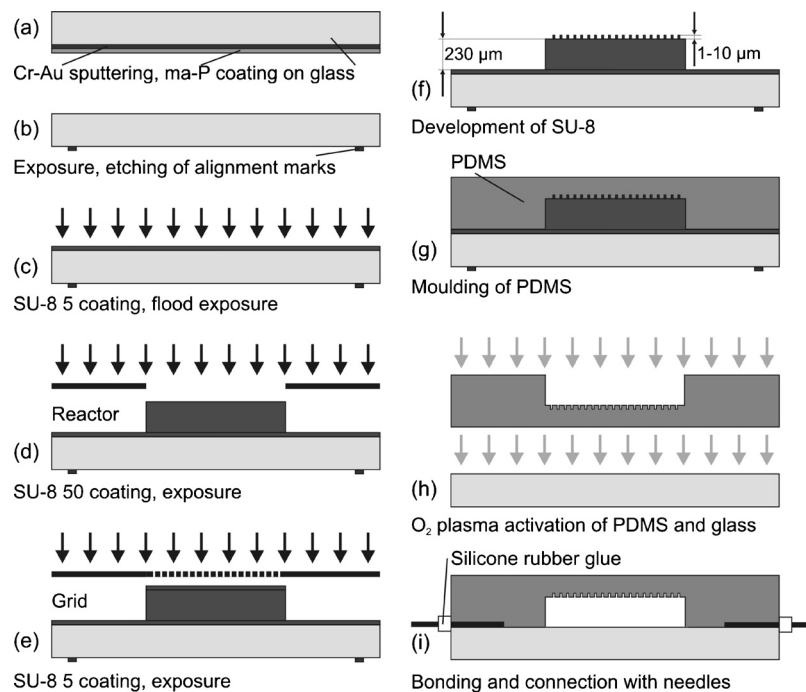


FIG. 2. Fabrication procedure of the microfluidic device made of PDMS and glass via double lithographic processing: fabrication of the SU-8 negative master [(a)–(e)], PDMS molding, bonding, and final assembling [(f)–(h)].

obtained. After the postexposure bake for 45 min at 95 °C, unpolymerized regions of the SU-8 are removed in propylene glycol methyl ether acetate (MicroChem Corp., Newton, MA, USA) [Fig. 2(f)]. Replicas of this master are patterned by using PDMS (Sylgard 184 elastomer kit, Dow Corning, Midland, MI, USA) in the standard ratio of 10:1 (silicon elastomer base: curing agent), which is heated to 80 °C for 30 min [Fig. 2(g)]. The structured PDMS elements are irreversibly bonded to a glass chip after oxygen plasma activation (plasma activate flecto 10 USB, Plasma Technology, Rottenburg, Germany) [Fig. 2(h)]. Finally, needles (Ø of 0.8×40 mm, BRAUN Sterican, Melsungen, Germany) are inserted into the inlet and outlet channels of each reactor chamber and sealed with biocompatible silicone rubber glue (RS Components 692-542, Mörfelden-Walldorf, Germany) [Fig. 2(i)].

B. Strain and spore preparation

The applied model spores belong to the strain *Aspergillus ochraceus* Wilhelm (DSM 63304). Preparation of this spore suspension was performed as follows. Spores were grown on 30 g/l malt extract (Becton, Dickinson and Co., Le Pont de Claix, France), 3 g/l soya-peptone, and 15 g/l agar (both Fluka, Steinheim, Germany) at 24 °C. Conidia were harvested after 8 days by flooding the agar surface with 25 ml sterile 0.9% NaCl (Carl Roth, Karlsruhe, Germany) solution. The spore suspension was filtered (miracloth filter, pore size of 25 µm, Calbiochem, La Jolla, CA, USA) to remove agar residues and hyphae. The concentration of existing spores was obtained by measuring the optical density of the suspension (SmartSpec™ 3000, BioRad, München, Germany).²⁵ Preliminarily, a linear correlation between spore concentration and specific extinction at a wavelength of 600 nm was determined by applying different dilution series of spore suspensions which were counted by means of a Thoma chamber.

C. Cultivation procedure for spore germination screening

The cultivation medium of *A. ochraceus* spores contained 30 g/l malt extract and 3 g/l soya-peptone. For germination studies, 5 ml of this malt extract liquid medium (ME medium) was inoculated with the spore suspension to an initial spore concentration of 1×10^6 mL⁻¹. Before inoculation the desired pH was adjusted (pH 3.5, 5.5, and 7.5).

Each reactor chamber of the microbioreactor was preliminarily disinfected with 70% ethanol for 2 min. To evaporate the ethanol, the microdevice was incubated at 105 °C for 5 min. Each reactor chamber was rinsed with sterile ME medium and finally filled with 100 µL of the inoculated suspension manually via a syringe. The open ends of the needles were finally connected to Luer-lock-adapters (Novodirect GmbH, Kehl, Germany) which again were joined to tubing. All ends of the inlet and outlet tubing were connected to a glass flask containing 30 ml of sterile ME medium. This flask was located 30 cm above the microbioreactor in order to induce a hydrostatic force inside the incubation chamber and avoid dehydration of the chamber. Bubble formation within chambers of small volume in microdevices is undesirable and can be avoided by induced overpressure.²⁶ Due to increased hydrostatic pressure, potentially generated bubbles are forced to permeate through the gas permeable PDMS membrane. The microdevice was then located under the optical microscope (Axioskop, Zeiss, Jena, Germany) with its glass side downward and its PDMS cover upward to ensure the oxygen supply during germination. To keep the cultivation temperature constant at a value of 24, 26, 28, 30, 32, and 37 °C, a temperature controlled incubation chamber was developed which is constructively adapted to the microscope dimensions. It consists of a temperature controller (KT4, Panasonic, Osaka, Japan), a switch cabinet heating system (RO/SE, Bad Birnbach, Germany), and a Peltier element (Quick-Cool QC 127-1.4-8.SMD, Quick-Ohm Küpper, Wuppertal, Germany). This setup allows temperature stabilization of ± 0.1 °C. During germination images were taken at the different locations in defined time intervals with the integrated microscope camera (AxioCam MRc, Zeiss, Jena, Germany) and a 20× zoom lens. Figure 3 shows the assembly of the microbioreactor chip, the complete setup under the microscope (incubation chamber box not shown), and the grid matrix with germinated spores after 9 h.

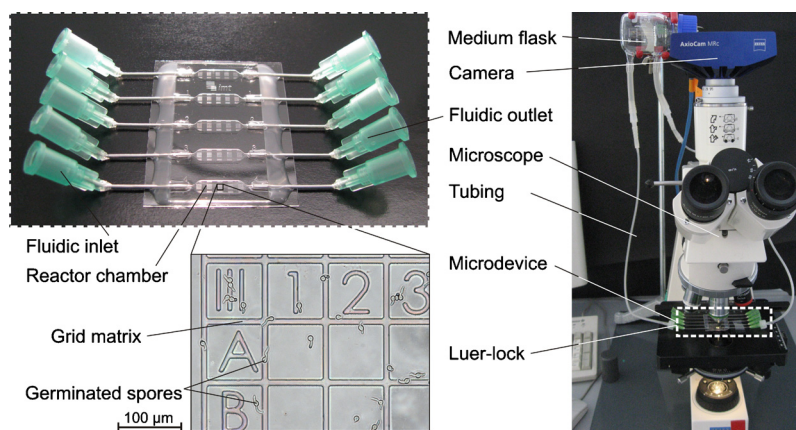


FIG. 3. Assembled microdevice with the parallel microchambers and an enlarged image of the grid matrix with germinated spores (left), complete setup for germination screening (incubation chamber box not shown) (right).

D. Shake flask cultivation

In order to validate the microreactor application and the down-scalability from conventional laboratory systems, the time course of biomass growth in shake flasks was determined. Therefore, cultures of *A. ochraceus* grown in 100 ml baffled flasks containing 25 ml ME medium (pH 3.5, 5.5, 7.5) were inoculated with the spore suspension to an initial spore concentration of $1 \times 10^6 \text{ mL}^{-1}$ and subsequently incubated for 46 h at 24 °C and 130 min^{-1} on a rotary shaker (Certomat BS-1/50 mm, Sartorius, Germany). Cell dry weight (CDW) was determined gravimetrically (CD 225 D, Sartorius, Göttingen, Germany). Therefore, 5 ml of a sample was filtered on a cellulose filter (Filter Disks, Grade 389, Sartorius, Germany). The filter was then washed twice with de-ionized water and dried at 105 °C for 48 h. After cooling in a desiccator, the CDW (g/l) was determined by reweighing. All values represent the average of triplicate trials.

IV. RESULTS

The microbioreactor device was used for parameter studies with regard to germination behavior of fungal spores using the example of *A. ochraceus* as a model organism. For evaluation of all screening results in the microdevice, the percent germination, being defined as the percentage ratio of already germinated spores to the total number of spores, was plotted versus the incubation time. In doing so, the germination behavior in dependence of different environmental conditions such as pH and temperature could be estimated and compared to the results obtained in conventional laboratory systems to confirm the cultivation protocol. This visually determined dependency offers valuable information about the viability and germination capability of the conidia of *A. ochraceus* under submerged conditions.

A. Germination versus cultivation time

Figure 4 illustrates a typical germination characteristic of *A. ochraceus* in the microbioreactor, exemplified for conditions of pH 5.5 at a temperature of 24 °C in ME medium. These parameters were recommended by the DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). The three basic structural modifications during germination process can be microscopically observed: swelling of conidia, emergence of germ tubes, and elongation of hyphae. During the first hours, the spores adapt themselves to the conditions in the microbioreactor, which is also called the activation period.⁷ Due to water uptake and growth of spore wall, a first morphological change is observed from 0 to approximately 8 h, also referred to as swelling. After 9 h, the volume of each spore has increased approximately four times in comparison to the initial volume of the dormant conidia, and some of the spores begin to form the first germ tubes.

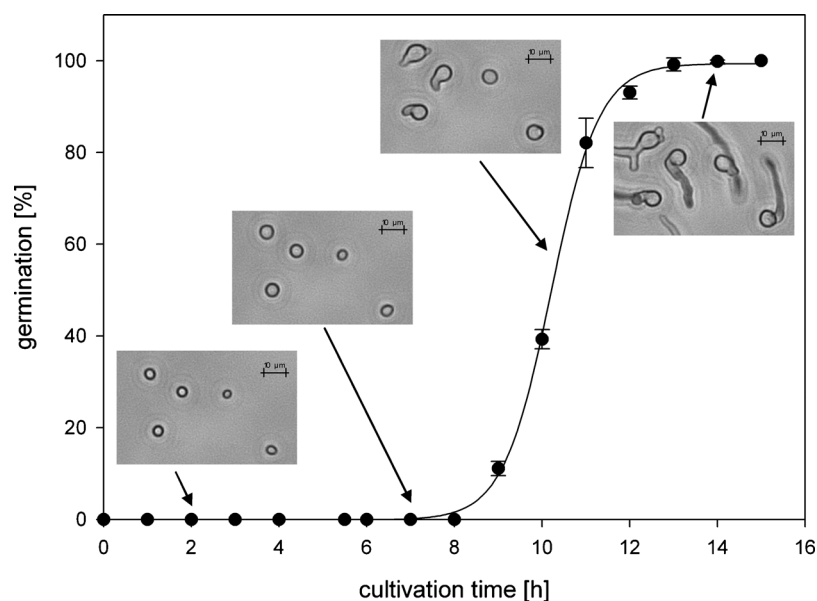


FIG. 4. Germination of *A. ochraceus* during 20 h of incubation at 24 °C in ME medium (pH 5.5): percent germination over cultivation time.

This state can be characterized as the polarized growth since wall deposition becomes polarized. After 15 h, almost all spores (98%) have germinated. The vegetative growth begins as per definition when the tube length is as long as the width of the germ tube.⁶ By then, the germ tubes continue to elongate resulting in branching mycelium. It could be observed that most of the dormant spores of *A. ochraceus* generated one to occasionally two germ tubes.

In the following, the germinability is analyzed under different environmental conditions (pH and temperature) and compared with the results attained in flasks and on agar plates.

B. Screening of pH

Conidial fungi can grow over a wide range of pH. Most tolerate a pH range from 4 to 9 but grow and germinate optimally near neutral pH.²⁷ Miles and Trinci²⁸ showed that especially the wall thickness of the spores and the length of the hyphal growth unit are very sensitive to pH changes when studying the morphology of *P. chrysogenum*.

For the pH screening, each of the chambers on one microchip is filled with the same inoculum but different medium varying in the pH value (3.5, 5.5, and 7.5). Figure 5 traces the germination curves for the different pH values. Data are given in mean values and standard deviations are indicated for three replicates. The percentage of germination was determined after 5.5, 9, 11, and 15 h and evaluated visually by counting approximately 130–150 conidia (total value of five pictures) in each microchamber.

It can be seen that germination is promoted for a pH of 5.5 resulting in a 3.5 times higher germination (25%) after 9 h when compared to a pH of 3.5 (8%) and 7.5 (6%). This difference even increases for a cultivation time of 11 h, where 85% of the spores in the pH 5.5 medium have already germinated, whereas in the pH 3.5 medium the percent germination only reaches a value of 11%. Abdel-Rahim *et al.*⁸ also identified that spores of *Aspergillus niger* were very sensitive to changes in the hydrogen ion concentration. They even reported on a complete inhibition of germination at a pH smaller than 3.5. After 15 h of incubation, all conidia in the pH 5.5 medium are germinated and an elongated and branched mycelium can be observed. For higher and lower pH values, the germination rate only reaches a value of 95% and the conidia generally show a slower hyphal growth. To conclude, a pH of 5.5 seems to be optimal for *A. ochraceus* with regard to rapid germination and thus high space-time yield of biomass in production scale.

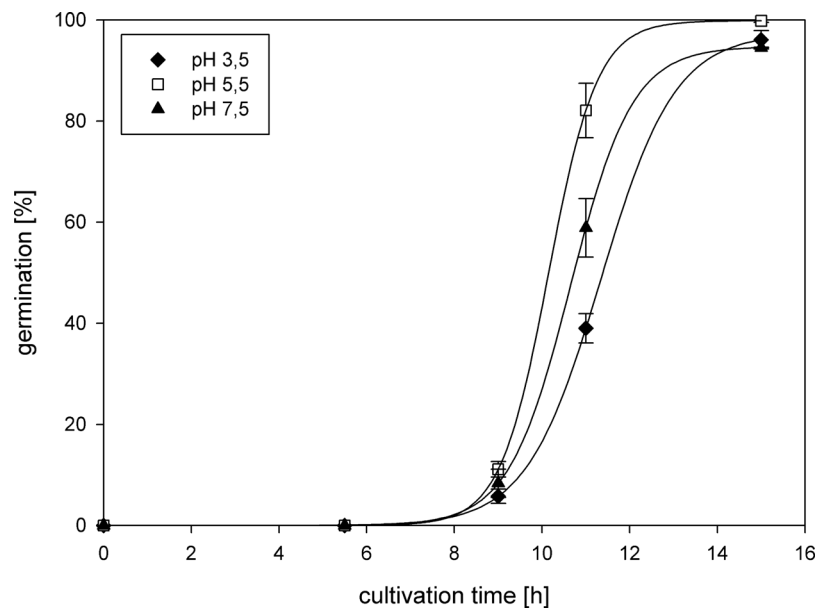


FIG. 5. Effect of different pH values (pH 3.5, 5.5, and 7.5) on the germination capability of *A. ochraceus* for the cultivation time of 15 h. Each time course of germination represents the average of triplicate experiments. Error bars show standard deviations.

In order to prove the down-scale feasibility of the applied microchip, pH screening was also performed in shake flasks. Figure 6 illustrates the time course of the CDW (g/l) for same pH values and same species in 25 ml culture suspension in 100 mL shake flasks. Most rapid biomass growth behavior is again obtained for a pH of 5.5 and thus showing the same results also found within the PDMS microchip. The main drawback of cultivation in shake flasks as an alternative

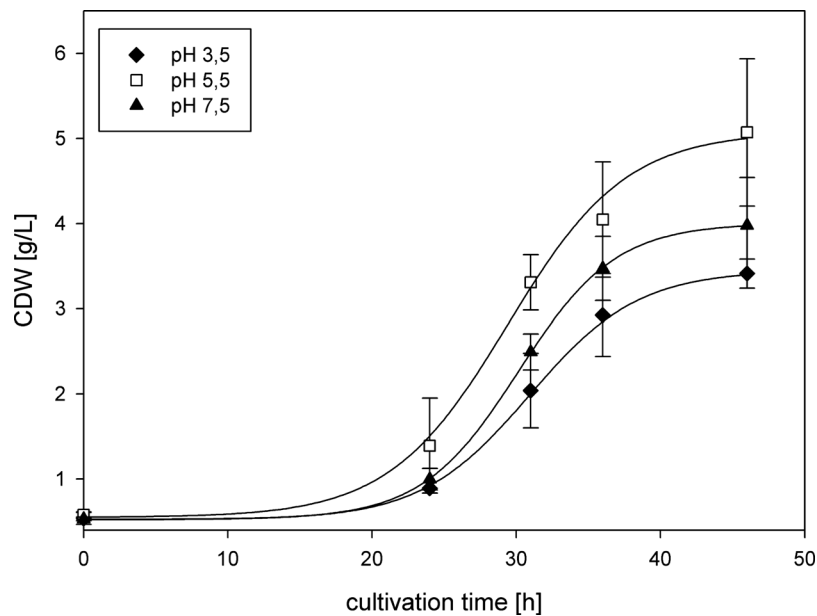


FIG. 6. Effect of different pH values (pH 3.5, 5.5, and 7.5) on the biomass growth of *A. ochraceus* in 100 ml shake flasks for the cultivation time of 46 h. Each time course of CDW represents the average of triplicate experiments. Error bars show standard deviations.

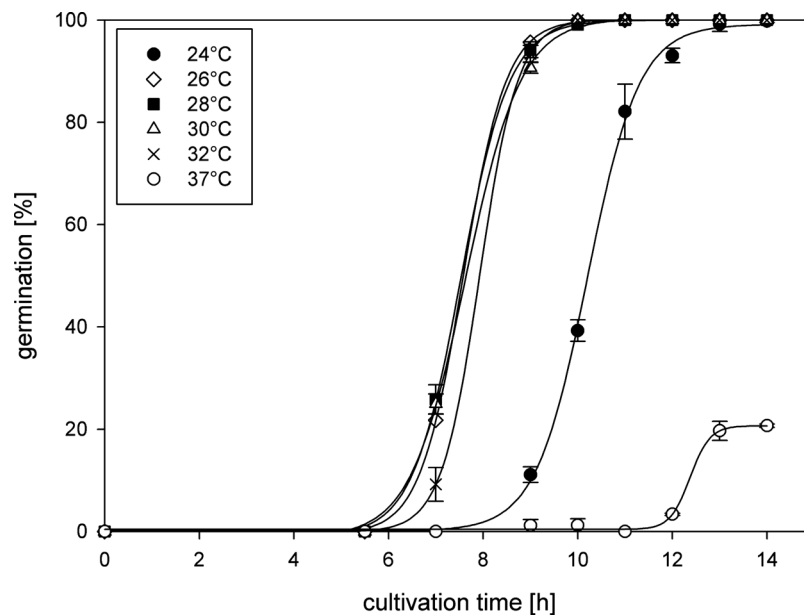


FIG. 7. Effect of different temperature screening (24, 26, 28, 30, 32, and 37 °C) on the germinability of *A. ochraceus* for a cultivation time of 14 h. Each time course of germination represents the average of triplicate experiments. Error bars show standard deviations.

conventional method is that the determination principle is limited to the CDW. Of course, the first germination effect already takes place within 6–10 h. However, during this period, no reliable measuring techniques are provided. Optical density measurements with a spectrometer can be excluded due to the filamentous hyphen formation. Another possibility would be to take samples by means of a pipette and counting them on a microscope slide. However, this is not reliable, as for each interval sampling new volume has to be taken, and furthermore the spores are exposed to stresses during pipetting. The last method is based on gravimetry. However, this method cannot be applied within 6–10 h of the germination process because the gravimetric change is not detectable. Therefore, the only possibility is to measure the CDW after a longer timer interval (20–50 h). However, both parameters—the CDW and the percent germination for shake flasks and the microdevice—describe the same growing performance of *A. ochraceus*, because faster germination rates will lead to higher biomass concentration. The percent germination describes the early growing, whereas the CDW points out the further growth devolution. Therefore, screening performance in the microdevices is validated.

C. Screening of temperature

Studies of temperature effects on growth and metabolite production are few with respect to filamentous fungi. However, the quantity of germinated spores and the time of germination are also found to be influenced by the incubation temperature. In Fig. 7 each germination curve represents the average of triplicate experiments. About 140 ± 10 spores per time point and micro-chamber were measured for each experiment. The pH value of culture medium was set constant to the optimal value of 5.5 concluded from the preliminary experiments.

Distinct germination patterns were found at various temperatures. In the range of 26–30 °C, germination capacity does not differ significantly. In this temperature range, germination is enhanced, resulting in almost 60% of germinated spores after 8 h. Complete germination is obtained after 10 h. At the same time, the spores cultivated at 24 °C only demonstrate 40% germination. For higher temperatures the growth declines further. At 37 °C only 20% of the spores have

germinated after 14 h. To conclude, an optimal temperature with regard to rapid germination under submerged conditions was found to be between 26 and 30 °C.

Roberts *et al.*²⁹ also reported on a temperature optimum between 24 and 30 °C for *A. ochraceus* when growing the spores on solid surface (agar plates) under atmospheric conditions in a range between 8 and 37 °C. This implies again that the presented reactor design is comparable to conventional plate count technology. However, it is now well accepted that agar plates are limited for germination screening, because this method will only express the proportion of population that will start germination under selected culture conditions.³⁰ Moreover, germination studies on solid agar plates are often time consuming and require up to 3–4 days. The presented biomicroreactor device gives information about germinability in only 14 h.

Since the average standard deviation for all performed experiments is only 0.9% for triplicate experiments, the developed microdevice presents a disposable, inexpensive tool for reliable data analysis, which is comparable to conventional laboratory systems. However, so far all results were obtained by visual counting method in order to, first of all, ensure the feasible application of the developed PDMS microdevice for germination screening.

To conclude, the microdevice developed here offers fungal physiologists a new tool to inexpensively and rapidly screen spore germination characteristics and its successive influence on cultivation productivity. Furthermore, it could be applied for fundamental research considering the influence of different genes on the morphology of filamentous fungi in submerged conditions while replacing the traditional plate count technique.

V. CONCLUSION

In this work, the potential of disposable, inexpensive microdevices for rapid morphology screening could be successfully proven. In order to establish quality indicators for cultivation performance of different fungi species, for example, the use of microbioreactors for germination screening shows different advantages when compared to the conventional determination methods. The PDMS microdevice containing five identical, parallel reactor chambers presents a noninvasive technique to continuously monitor the different germination behaviors of submerged cultivated spores (such as swelling characteristics, germ tube formation, and extension kinetics) with an ensured oxygen supply over time. These germination characteristics are potentially valuable in studies of the quality of initial spore preparations and proposed cultivation media. Therefore, in order to validate the cultivation protocol in microdevices and the down-scalability from laboratory scale systems, germination screening of a model organism (*A. ochraceus*) for two different process conditions (pH and temperature) was carried out. Optimal germination parameters resulted to be a pH of 5.5 and a temperature ranging from 26 to 30 °C. According to up-scaling characteristics, same optimal pH values could also be proven for biomass characteristics in shake flasks. Equal temperature ranges for cultivations of same species on agar plates were reported in literature that proves the application of the device. Consequently, screening within these microchips is less time consuming (1 day instead of 3–4 days) and less cost consuming (no elaborate sampling necessary) than parameter studies within flasks or on agar. With regard to screening in gridless microwell plates, no bulky and expensive equipment (inverted microscope with automatic stage) is required. In addition, only minimal amounts of culture medium are needed. Another advantage of the microchip is its possibility of continuous cultivation. Therefore, the analysis of, e.g., any additives that can be homogeneously distributed within the reaction chamber during germination can easily be screened. This work represents a clear step toward an increasing impact of microtechnologies and microfluidics on biotechnological research.

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